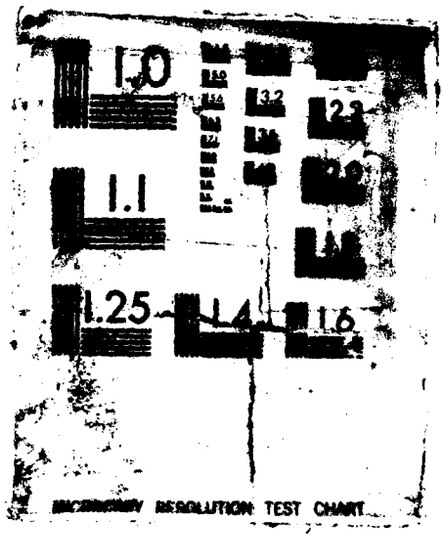
AD-A184 308 USE OF DNA PROBES FOR DIAGNOSIS OF INFECTIOUS DISEASES 1/1
(U) CALIFORNIA UNIV OAKLAND NAVAL BIOSCIENCES LAB
D T KINGSBURY 1987 UC-NBL-970 N00014-81-C-0570
UNCLASSIFIED F/G 6/5 NL


1.0
1.1
1.25
1.4
1.6
RESOLUTION TEST CHART

SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY

(2)

REPORT DOCUMENTATION PAGE

DTIC ELECTED SEP 09 1987 S D

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS: NONE
2a. SECURITY CLASSIFICATION AUTHORITY: N/A	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE: N/A	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): NBL No. 970	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of California; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Naval Biosciences Laboratory, Naval Supply Center, Oakland, California 94625	7b. ADDRESS (City, State, and ZIP Code): Code 1141, 800 North Quincy Ave, Arlington, VA 22217-5000
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-81-C-0570
8c. ADDRESS (City, State, and ZIP Code): 800 North Quincy Avenue, Arlington,Va 22217-5000	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
61153N	RR041-05	RR041-05-03	NR204-123

11. TITLE (Include Security Classification): (U) USE OF DNA PROBES FOR DIAGNOSIS OF INFECTIOUS DISEASES

12. PERSONAL AUTHOR(S): Kingsbury, David T.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Summary Report	FROM 861001 TO 870930	1987	5

16. SUPPLEMENTARY NOTATION: In Press. 1987

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Keywords: DNA Hybridization, Modern Molecular Biology,
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DNA hybridization is well established as an essential tool in modern molecular biology. In the past few years an increasing number of investigators have applied this technique to the detection and identificaton of a variety of infectious agents. A review of the scientific literature suggests that at the present time there are more than 20 DNA probes suitable for diagnostic purposes. The limiting factor in their full implementation as a diagnostic test is the development of a supporting 'system', including suitable procedures for sample preparation and a high specific activity, non-radioactive DNA tagging procedure.

AD-A184 308

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Head, Biological Sciences Div, ONR	(202)696-4986	ONR Code 1141

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

970

USE OF DNA PROBES FOR DIAGNOSIS OF INFECTIOUS DISEASES

David T. Kingsbury
Assistant Director
Biological, Behavioral and Social Sciences
National Science Foundation
USA

DNA hybridization is well established as an essential tool in modern molecular biology. In the past few years an increasing number of investigators have applied this technique to the detection and identification of a variety of infectious agents. A review of the scientific literature suggests that at the present time there are more than 20 DNA probes suitable for diagnostic purposes. The limiting factor in their full implementation as diagnostic tests is the development of a supporting "system", including suitable procedures for sample preparation and a high specific activity, non-radioactive DNA tagging procedure.

An historical view.

The origins of modern DNA probe technology can be traced back to the early work on the physical properties of DNA itself. Early workers observed that the adsorbance of a DNA solution at 260 nm increased by approximately one-third upon boiling and would then begin to decrease again if the DNA was cooled very slowly, whereas this would not occur if the DNA was rapidly cooled. The apparent explanation for this was the reassociation of the separated DNA strands under the slow cooling conditions. The conditions which controlled this process were extensively examined by Britten and Kohne (1968) and Wetmur and Davidson (1968).

Many investigators recognized the potential of using this technique for the determination of the genetic and taxonomic relationships between organisms, principally microorganisms. The power of nucleic acid hybridization as a tool for the detection and identification of infecting microorganisms was first fully realized in the study of tumor virus transformed cultured animal cells. The potential for the quantitative estimation of the number of viral copies as well as the ability to determine the fraction of the viral genome present brought this technique into common use.

The introduction of recombinant DNA technology had a two fold impact on DNA probe development. First, it enabled investigators to prepare large quantities of specific DNA fragments which could be employed

as probes for the examination of the biology and biochemistry of those specific genetic sequences. Secondly, hybridization and probe technology became central to the identification and characterization of cloned DNA fragments. The development of the Southern blot procedure (Southern, 1975) revived the use of nitrocellulose filters as a hybridization matrix and led to a series of techniques for the filter analysis of cloned DNA sequences and more recently clinical and environmental samples.

The fundamental concepts behind nucleic acid probe applications.

Nucleic acid probe hybridization provides a convenient method for the detection and measurement of specific defined nucleotide sequences in a mixture of heterologous sequences. The reaction rate between homologous sequences is dependent upon their concentration, the salt concentration and the incubation temperature. Figure 1, is a schematic representation of the application of DNA probes to the detection of a microorganism.

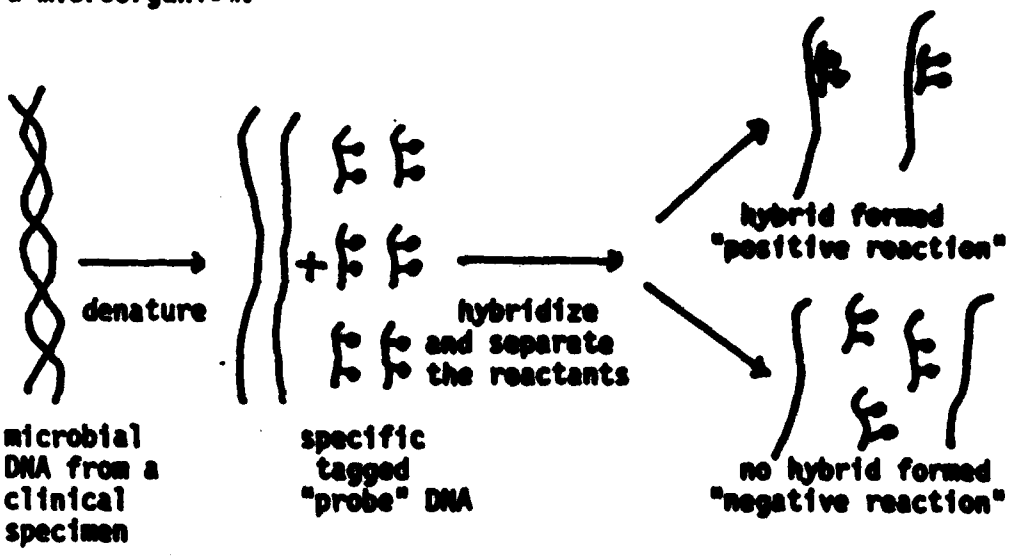


FIGURE 1. Steps in the identification of specific nucleotide sequences through DNA probe hybridization.

This schematic diagram highlights several of the important elements of the hybridization procedure. The principal areas of interest in the continued development and commercialization of this procedure are: (1) design and production of the probe; (2) tagging the probe for easy detection; (3) preparation of the sample to be examined; (4) the hybridization conditions themselves; and (5) separation of the reacted from the unreacted products and their subsequent analysis.

The philosophy of diagnostic probe design and development.

Probes may be either RNA or DNA and should be no less than 25 bases in length in order to form a stable native hybrid. Depending upon the exact application, probes usually range from 30 bases to 5 or as high as 10 kilobases in size. To be optimally effective the probe must share substantial nucleotide sequence homology with the sequences to be detected; however, total nucleotide sequence homology is not essential. A mismatch of as much as 25 % to 30 % is tolerated in many probe applications, although the hybridization conditions must be adjusted when significant mismatching is anticipated.

The design of probes for the identification of infectious agents may take several directions. Generally, probes are designed to specifically identify a given microorganism of interest to the exclusion of all others. There are many examples of this approach. In my laboratory we have been interested in a balanced approach to the detection and identification of a group of bacteria, the mycoplasmas, which are of considerable medical and commercial significance and are difficult, or often impossible, to cultivate in vitro. Because of the genetic heterogeniety of these organisms, where even those species thought to be closely related only share 10 % to 15 % nucleotide sequence homology, the isolation of a species specific probe for each of the two most medically significant species, Mycoplasma hominis and Mycoplasma pneumoniae, required only limited screening of a library of recombinant clones. In contrast, the derivation of species specific probes within the enteric bacteria and the bacilli has been extremely frustrating and labor intensive. The difficulty in dealing with these latter groups stems from their high degree of nucleotide sequence conservation between species.

In some instances it has been possible to use genes encoding known virulence factors, such as toxins, as probes for microorganisms. Falkow and Moseley (1980) employed the genes for the enterobacterial heat labile toxin (LT) and the heat stable toxin (ST) in epidemologic examinations of outbreaks of bacterial diarrhea. In this instance it was possible to identify the pathogenic strains within a background of the same and closely related species which were not pathogenic. A similar approach has been possible with the plague bacillus (Portnoy et al., 1983). Recent work by Perine et al. (1985) demonstrated the utility of DNA probe diagnosis using a combination of a species specific probe for Neisseria gonorrhoeae and a probe specific for the beta-lactamase plasmid. This approach led to the simultaneous assessment of the presence of the microorganism and whether or not it contained the penicillinase gene.

The selection of tags for nucleic acid probes.

In the past three years it has become very clear that the eventual commercialization of nucleic acid probes as diagnostic tools is dependent upon the replacement of radioactive labels with easily detected, long shelf-life, non-radioactive tags. The most commonly employed tag currently is phosphorous-32 incorporated into the probe by enzymatic polymerization of nucleoside phosphates. This is an

excellent tag for most research applications where a short half-life and the hazards of radioactive materials are not major concerns. In less sophisticated settings however, a new approach is needed. There have been, and continue to be, serious attempts to find better probe labeling techniques. The use of antibodies which specifically recognize double stranded DNA or RNA:DNA hybrids, the use of biotinylated nucleotides, the introduction of photon transfer systems and the direct coupling of enzyme tags on probes are also being examined. There seems little question that the eventual commercial success of probe diagnostics is tied to the development of a suitable (detection) "system".

Sample preparation and separation technology.

It may seem strange to group together these two steps of the total process; however, at the moment they are very closely linked. One of the powerful attributes of probe technology is the ability to make quantitative determinations based on hybridization kinetics. This is most feasible when using solution hybridization in contrast to reactions done on a solid matrix. Solution hybridization, however, introduces constraints on sample preparation and requires the separation of the reacted from unreacted probe by a procedure such as hydroxyapatite chromatography. A far more convenient sample preparation system involves the placing of the sample on a solid support, such as a nitrocellulose filter, and the partial purification and denaturation of the DNA on that matrix which is then used for the hybridization reaction. Separation of the reacted from unreacted probe is achieved by simply washing the filter. It is the filter system that is in the most widespread use currently; however, it is clear that the application of this procedure to clinical specimens is fraught with dangers. We, and our colleagues at Stanford, have noted that many clinical materials have interfering substances which introduce abnormally high probe non-specific binding or prohibit binding altogether. It is very clear that far more attention must be paid to the nature of the starting sample in order for this technology to be widely applicable clinically.

The importance of hybridization reaction conditions.

The continuing challenge to the further development of nucleic acid probe technology outside of the research environment is to make it simple and to make it fast. Diagnostic tests must not take days to give results but should be run in minutes to hours. Factors in the hybridization equation which can be manipulated are the conditions of salt, temperature and DNA concentration used in the hybridization.

The most easily manipulated parameter of the hybridization system is the concentration of the reactants. In our experience the most significant factor which allows us to rapidly detect microorganisms is to increase the concentration of the probe such that it is the factor driving the reaction. This might mean probe concentrations approaching 1 mg/ml in the actual hybridization reaction. It is our estimate, and this is shared by several other workers in the field, that a four hour probe diagnostic test is currently realistic and that a two hour test is not unattainable.

REFERENCES

1. Wetmur, J.G. and N. Davidson, J. Mol. Biol. 31:349, (1968).
2. Britten, R.J. and D.E. Kohne, Science 161:529, (1968).
3. Southern, E.M., J. Mol. Biol. 98:503, (1975).
4. Falkow, S. and S.L. Moseley, U.S. Patent 4,358,535, (1980).
5. Portnoy, D.A. et al., J. Infect. Dis. 148:297, (1983).
6. Perine, P.L., et al., J. Infect. Dis. 152:59, (1985).

Professor Kingsbury is currently on leave from the University of California, Berkeley where he holds the position of Professor of Medical Microbiology and Virology in the School of Public Health and is Director of the Naval Biosciences Laboratory.

The research reported here was supported by the Office of Naval Research.

END

9-87

DTIC